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(54) Title: COMPOUNDS AND METHODS FOR CHEMOSENSITIZING MULTIDRUG RESISTANT CELLS

(57) Abstract

Compounds and methods for treating multidrug resistant cells are disclosed which are useful in cancer therapy.

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COMPOUNDS AND METHODS FOR CHEMOSENSITIZING MULTIDRUG RESISTANT CELLS

This invention was made with government support under Grant CA64983 awarded by United States Public Health Service. The government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to cancer therapy. More particularly, this invention relates to chemical compounds and compositions possessing multidrug resistance reversing activity, and to methods of chemosensitizing multidrug resistant cells using such compounds and compositions.

BACKGROUND OF THE INVENTION

Current anticancer drugs often provide temporary clinical improvement; however, the emergence of tumor cells that are resistant to the original agent and other structurally unrelated drugs often results in the ultimate failure of chemotherapy. A common mechanism for this acquisition of multidrug resistance (MDR) involves the overexpression of transport proteins that efficiently efflux most natural product anticancer drugs. The biochemistry and pharmacology of the prototypical drug transporter, P-glycoprotein, has been intensely studied, and similar studies are progressing with the more recently discovered multidrug resistance-related protein (MRP).

In 1981, verapamil was shown to reverse MDR in tissue culture, setting off intensive searches for clinically useful MDR modulators. Subsequently, numerous compounds have been shown to reverse P-glycoprotein-mediated MDR in cell culture, usually promoting drug accumulation and cytotoxicity by

competing with the cytotoxic drugs for binding to P-glycoprotein. Several clinical trials using some of these agents, e.g., verapamil, dexverapamil, other calcium channel antagonists, cyclosporine compounds, or certain steroids, as modulators of MDR have been described. Unfortunately, the clinical success with the modulators used heretofore has been unimpressive, predominantly due to their intrinsic toxicity and their untoward effects on the pharmacokinetics of the accompanying anticancer drugs.

The lack of successful modulation of MDR in the clinic may result from incomplete knowledge of the roles and pharmacologies of multiple transport proteins. It may therefore be useful to compare the known properties of P-glycoprotein and MRP. One significant difference between P-glycoprotein and MRP relates to their distributions in normal tissues. P-glycoprotein has been shown to be expressed by several types of secretory cells, such as capillary endothelial cells in the brain and testis, and at sites within the pancreas, kidney and liver. In contrast, the expression of MRP mRNA occurs in virtually every type of tissue. Significantly, peripheral blood mononuclear cells are prominent sites of MRP expression.

Tumor cells from patients undergoing chemotherapy often demonstrate elevated P-glycoprotein expression, suggesting that this mechanism of MDR is clinically important. Recent studies have indicated that MRP is expressed in a high percentage of solid tumors and leukemias. However, no differences in MRP levels were detected between normal and malignant hematopoietic cells, and MRP levels were found to be lower in lung tumors than in normal lung tissue. Chemotherapy did not alter the expression of MRP in malignant melanoma, acute lymphocytic leukemia or chronic lymphocytic leukemia, but promoted modest increases in MRP mRNA levels in acute myelogenous leukemia. Therefore, it seems that

overexpression of P-glycoprotein activity is of greater clinical significance than elevation of MRP. Inhibition of MRP by MDR modulators is likely to increase the uptake of cytotoxic anticancer drugs by normal tissues, thereby producing greater systemic toxicity.

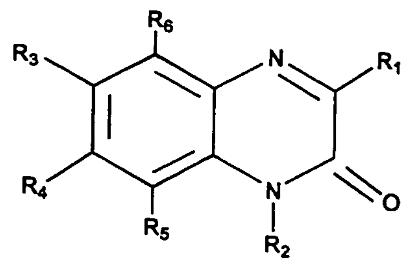
Another important consideration of P-glycoprotein and MRP relates to their drug specificity. Pharmacological comparison of cells overexpressing either P-glycoprotein or MRP have demonstrated that the resistance profiles conferred by these two transporters are only partially overlapping. For example, MRP-transfected cells demonstrate greater resistance factors for vincristine, etoposide and doxorubicin, than for vinblastine and paclitaxel, whereas P-glycoprotein-overexpressing cells are extremely resistant to the latter two drugs. Differential abilities of certain compounds to antagonize P-glycoprotein and MRP have also been suggested.

The foregoing observations have led to the conclusion that non-selective MDR modulators tend to increase toxicity to the concomitantly administered anticancer drug to normal cells, including hematopoetic cells. It is believed that compounds which exhibit selectivity in antagonizing P-glycoprotein and MRP should be superior clinical agents.

SUMMARY OF THE INVENTION

In accordance with one aspect, the present invention provides compounds and compositions which have been discovered to have MDR reversing activity and to methods of chemosensitizing multidrug resistant cells using such compounds and compositions.

Compounds useful in the practice of this invention include those having the following formulae, including their pharmaceutically acceptable salts:

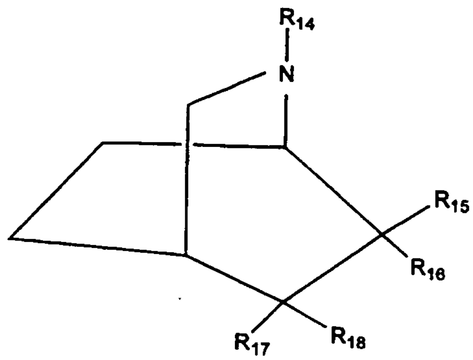


(I)

wherein R_1 represents substituted or unsubstituted phenyl (C_6H_5), the phenyl substituents being selected from the group consisting of alkyl (C_1-C_6), halogen, haloalkyl, -OH, -O-alkyl, hydroxyalkyl, carboxy, carbalkoxy, -SH, S-alkyl, mercaptoalkyl, $-NO_2$ and -NR'R", R' and R" being the same or different and representing H or alkyl (C_1-C_6);

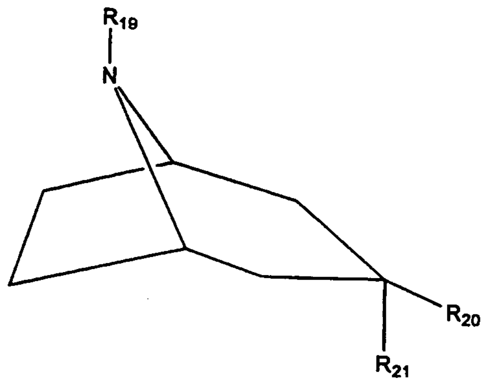
R_2 represents hydrogen or alkyl (C_1-C_6);

R_3, R_4, R_5 and R_6 may be the same or different and represent substituents selected from the group of H, alkyl (C_1-C_6), halogen, haloalkyl, -OH, -O-alkyl, hydroxyalkyl, carboxy, carbalkoxy, -SH, -S-alkyl, mercaptoalkyl, $-NO_2$ and -NR'R", R' and R" being as previously defined; or R_3 and R_4 taken together with the carbon atoms to which they are attached form an substituted or unsubstituted beneze ring (C_6H_4), the benzene ring substituents being at least one of R_3, R_4, R_5, and R_6, as previously defined; or



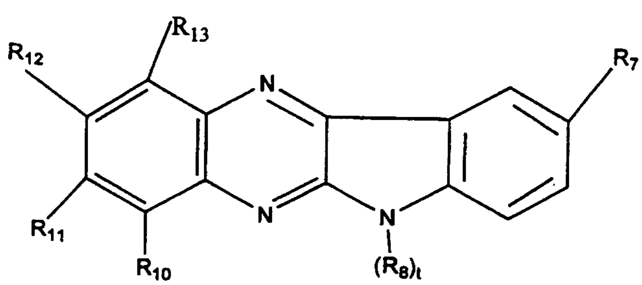
(II)

wherein R_{14} is selected from the group consisting of H, alkyl (C_1-C_6) or aralkyl (C_7-C_{11}), R_{15}, R_{16}, R_{17} and R_{18} may be the same or different and represent H, OH, hydroxyalkyl, -O-alkyl, -O-aralkyl, -SH, -S-alkyl, mercaptoalkyl, halogen, haloalkyl, substituted or unsubstituted, straight or branched chain alkyl (C_1-C_6), substituted or unsubstituted, straight or branched chain alkenyl (C_2-C_6), substituted or unsubstituted aryl (C_6-C_{10}), substituted or unsubstituted aralkyl (C_7-C_{11}), carboxy, carbalkoxy, and $-NR_aR_b$, R_a and R_b being the same or different and representing -H, alkyl (C_1-C_6), aryl (C_6-C_{10}) or acyl, or R_{15} and R_{16} together represent a carbonyl moiety (-C(=O)-), or R_{17} and R_{18} together with the carbon atom to which they are attached represent an alkyl substituted or unsubstituted alkylene ketal; the alkyl, alkenyl, aryl and aralkyl substituents being at least one of R_{15}, R_{16}, R_{17} and R_{18}, as previously defined; or



(III)

wherein R_{19}, R_{20} and R_{21} are the same or different and represent -H, -OH, -SH, alkyl (C_1-C_6), haloalkyl, -O-alkyl, hydroxyalkyl, hydrazino carbonyloxy, -S-alkyl, mercaptoalkyl, cycloalkylalkyl (C_4-C_{12}), substituted or unsubstituted aralkyl (C_7-C_{11}), or $-(CH_2-)_p$, NR_aR_b, R_a and R_b being as previously defined, and p=0 through 6, the aralkyl substituents being at least one of R_{19}, R_{20} and R_{21}, as previously defined, R_{19} additionally represents -C(=O)-O-R‴, wherein R‴ is p-chlorophenyl; or



(IV)

, wherein R_7, R_{10}, R_{11}, R_{12} and R_{13} are the same or

different and represent substituted or unsubstituted phenyl (C_6-H_5), the phenyl substituents being selected from the group consisting of alkyl (C_1-C_6), halogen, haloalkyl, -OH, -O-alkyl, hydroxyalkyl, carboxy, carbalkoxy, -SH, -S-alkyl, mercaptoalkyl, $-NO_2$ and -NR'R", R' and R″ being as previously defined, or R_{11} and R_{12} taken together with the carbon atoms to which they are attached form an unsubstituted or substituted benzene ring (C_6H_4), the benzene ring substituents being selected from the group of alkyl (C_1-C_6), halogen, haloalkyl, -OH, -O-alkyl, hydroxyalkyl, carboxy, carbalkoxy, -SH, -S-alkyl, mercaptoalkyl, $-NO_2$ and -NR'R", R' and R″ being as previously defined; and

R_8 represents H or alkyl (C_1-C_6), t=0 or 1.

Another series of compounds having MDR reversing

activity which may be used for chemosensitizing multidrug resistant cells in accordance with the present invention are the following:

V_1 - ethyl ester of 7-bromo-2-(3-chlorophenyl)-1,2,3,4-tetrahydro-8-methoxy-pyrazino[1,2-a]indole-10-carboxylic acid ($C_{21}H_{20}BrClN_2O_3$);

V_2 - ethyl ester of 5-(acetyloxy)-6-bromo-1-methyl-2-(4-morpholinylmethyl)-1H-indole-3-carboxylic acid, ($C_{19}H_{23}BrN_2O_5$);

V_3 - 2-chloro-N-N,β-trimethyl-12H-dibenzo[d,g][1,3,6]dioxazocine-12-propanamine ($C_{19}H_{23}ClN_2O_2$);

V_4 - 6-(4-bromophenyl)-3-methyl-1,4-diphenyl-1H-pyrazolo[3,4-b]pyridine ($C_{25}H_{18}BrN_3$);

V_5 - 1-cyclohexyl-3-(4-chlorophenylureido)pyrrolidine ($C_{17}H_{25}ClN_3O$);

V_6 - benzyl ester of 3,6-dihydro-4-(1-pyrrolidinyl)-1(2H)-pyridinocarboxylic acid ($C_{17}H_{22}N_2O_2$);

V_7 - N-phenyl-1-(phenylmethyl)-4-piperidinamine ($C_{18}H_{22}N_2$);

V_8 - methyl ester of 7-[1-(acetyloxy)-1-methylethyl]-1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-9-oxo,1-phenanthrenecarboxylic acid, [1R-(1α,4aβ,10aα)] ($C_{23}H_{30}O_5$);

V_9 - N-[p-(3-chloro-4-methoxyphenyl)-β-hydroxyphenethyl]-phthalimide ($C_{23}H_{18}ClNO_4$);

V_{10} - 2,6-dimethoxy-4-methyl-5-[3-(trifluoromethyl)phenoxy]-8-quinolinamine ($C_{19}H_{17}F_3N_2O_3$);

V_{11} - ethyl ester of α-[[(1,1-dimethylethyl)amino]sulfonyl]-3,4-dimethoxy-benzenepropanoic acid ($C_{17}H_{27}NO_6S$);

V_{12} - N-ethyl-N-phenyl-1-(2-phenylethyl)-4-piperidinecarboxamide ($C_{22}H_{28}N_2O$); and

V_{13} - 1-phenyl-2-oxo-6-phenethyldecahydro-1,6-naphthyridine ($C_{22}H_{27}N_2O$).

Also within the scope of this invention are isomeric forms of the above described compounds,

including, without limitation, cis- and trans-isomers of the compounds of formula II, and endo- and exo-conformations of the compounds of formula III.

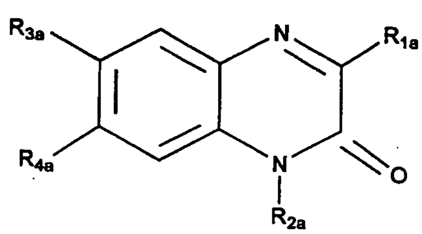
The above-described compounds may be formulated with a biologically compatible vehicle or carrier for use in reversing MDR and/or chemosensitizing multidrug resistant cells, as well as preventing MDR.

The present invention further provides methods for treating MDR, including reversing MDR, chemosensitizing multidrug resistant cells to anti-cancer agents, as well as preventing MDR, by administering an effective amount of at least one of the compounds described herein to a patient in need of such treatment.

The present invention provides compounds which are selective antagonists of either P-glycoprotein or MRP. Compounds which inhibit both drug transporters are also described. Therefore, these compounds and their use for chemosensitizing multidrug-resistant cells should provide important new therapies for drug-resistant cancers.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Compounds included in the foregoing summary have been found to potentiate the cytotoxicity of anticancer drugs. Particularly preferred are compounds of the formula:

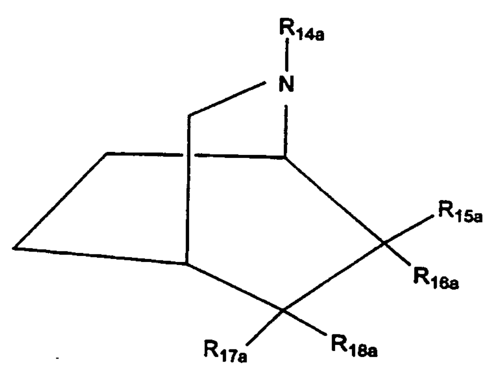


(IA)

wherein R_{1a} represents substituted or unsubstituted phenyl (C_6H_5), the phenyl substituents being selected from the group consisting of alkyl (C_1-C_6), -OH, -O-alkyl, halogen, and -NR'R″, R′ and R″ being as previously defined; R_{2a} represents H or alkyl (C_1-C_6); and R_{3a} and R_{4a} are the same or different and represent substituents selected from the group consisting of alkyl (C_1-C_6), -OH, -O-alkyl, halogen, and -NR'R″, R′ and R″ being as previously defined; or R_{3a} and R_{4a} taken together with the carbon atoms to which they are attached form a substituted or unsubstituted benzene ring (C_6H_4), the benzene ring substituents being at least one of R_{3a} and R_{4a}, as previously defined.

Representative examples of quinoxaline derivatives within Formula IA include, without limitation, 2-oxo-3-(2-amino-5-bromophenyl)-benz[g]quinoxaline (I-1_; 1-methyl-3-(2-methylamino-5-methoxyphenyl)-1,2-dihydrobenz[g]quinozalin-2-one (I-2); 1-methyl-3-(2-methylamino-5-methoxyphenyl)-1,2-dihydroquinoxalin-2-one (I-3).

Another particularly preferred group of compounds is that of the formula:

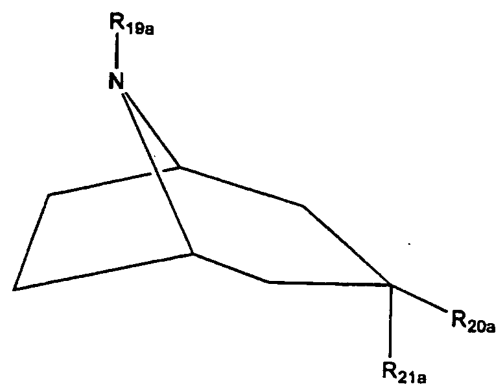


(IIA)

, wherein R_{14a} is selected from the group consisting of H, alkyl (C_1-C_6) or aralkyl (C_7-C_{11}), R_{15a}, R_{16a}, R_{17a} and R_{18a} may be the same or different and represent H, -OH, -O-alkyl, -O-aralkyl, substituted or unsubstituted, straight or branched chain alkyl (C_1-C_6), substituted or unsubstituted, straight or branched chain alkenyl (C_2-C_6), or substituted or unsubstituted aryl (C_6-C_{10}), or R_{15a} and R_{16a} together represent a carbonyl moiety (-C(=O)-), or R_{17a} and R_{18a} together with the carbon to which they are attached represent an alkyl substituted or unsubstituted alkylene ketal, the alkyl, alkenyl and aralkyl substituents being at least one of R_{15a}, R_{16a}, R_{17a} and R_{18a}.

Representative examples of azabicyclo[2.2.2]octane derivatives within Formula II_A include, without limitation, 2-phenethyl-5-oxo-2-azabicyclo[2,2,2]octane,1,2-(2,2-dimethyl)propylene ketal (II-1); 2-benzyl-cis-6-hydroxy-trans-6-(3,4,5-trimethoxyphenyl)-2-azabicyclo[2,2,2]octane (II-2); 2-benzyl-6-(3,4,5-trimethoxyphenyl)-2-azabicyclo[2,2,2]octane (II-3); cis-6-(3,4,5-trimethoxyphenyl)-2-azabicyclo[2,2,2]octane (II-4); trans-6-(3,4,5-trimethoxyphenyl)-2-azabicyclo[2,2,2]octane (II-5); 2-benzyl-cis-6-hydroxy-trans-6-(3,4-dihydroxyphenyl)-2-azabicyclo[2,2,2]octane (II-6); 2-ethyl-6-anilino-2-azabicyclo[2,2,2]octane (II-7); 2-phenethyl-6-(N-propionylanillino)-2-azabicyclo[2,2,2]octane (II-8); 2-benzyl-6-trans-hydroxy-2-azabicyclo[2,2,2]octane (II-9); 2-benzyl-6-(3,4-dibenzyloxyphenyl)-6-hydroxy-2-azabicyclo[2,2,2]octane (II-10); and 5-diphenylmethylidene-2-methyl-2-azabicyclo[2,2,2]octan-6-one (II-11).

A further particularly preferred group of compounds have the formula:



(IIIA)

, wherein R_{19a} is selected from the group consisting of H, alkyl (C_1-C_6), cycloalkyalkyl (C_4-C_{12}) or aralkyl (C_7-C_{11}), and R_{20a} and R_{21a} are selected from the group consisting of H, -NR_aR_b, R_a and R_b being as previously defined, or hydrazinocarbonyloxy.

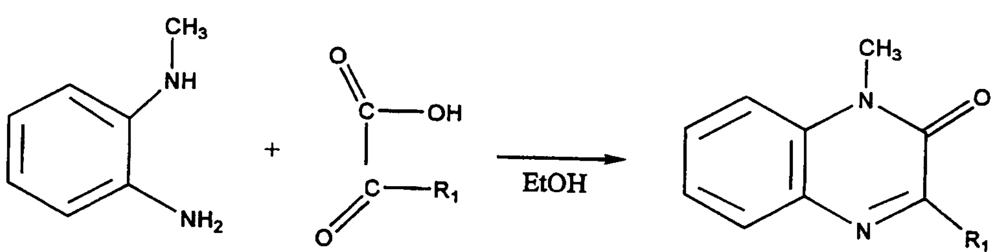
Representative examples of azabicyclo[3.2.1]octane derivatives within Formula III_A include, without limitation, 3-hydrazinocarbonyloxy-8-(4-chlorophenoxycarbonyl)-8-azabicyclo[3,2,1]octane (III-1); 3-(N-propionylanilino)-8-phenethyl-8-azabicyclo[3,2,1]octane (III-2); 3-(N-propionylanilino)-8-benzyl-8-azabicyclo[3,2,1]octane (III-3); and 3-(N-propionylanilino)-8-cyclopropylmethylene-8-azabicyclo[3,2,1]octane (III-4).

Representative examples of indolo[2,3-b]quinoxaline derivatives within Formula IV, above, include, without limitation, 1,4-dibutoxyindolo[2,3-b]quinoxaline (IV-1); benz[g]indolo[2,3-b]quinoxaline (IV-2); 5-methylbenz[g]indolo[2,3-b]quinoxaline (IV-3); and 6-methylindolo[2,3-b]quinoxaline (IV-4).

The quinoxaline derivatives used in the practice of this invention may, if desired, be synthesized according

to reaction schemes 1 or 2 shown below. Reaction scheme 1 is the more versatile of the two, in which N-methyl-1,2-phenylenediamine (or 1,2-phenylenediamine) is refluxed with an α-ketoacid derivative to directly produce 1-methyl-3-R_1-2-oxo-3,4-dihydroquinoxaline (or 2-oxo-3-R_1-3,4-dihydroquinoxaline) by condensation, R_1 being as previously defined. This reaction will occur with virtually any dicarbonyl compound, allowing the rapid synthesis of very diverse families of quinoxaline derivatives. A large number of starting materials, including variously substituted α-ketoacids, as well as mononuclear and polynuclear diamines, are available from Aldrich Chemical and other chemical suppliers.

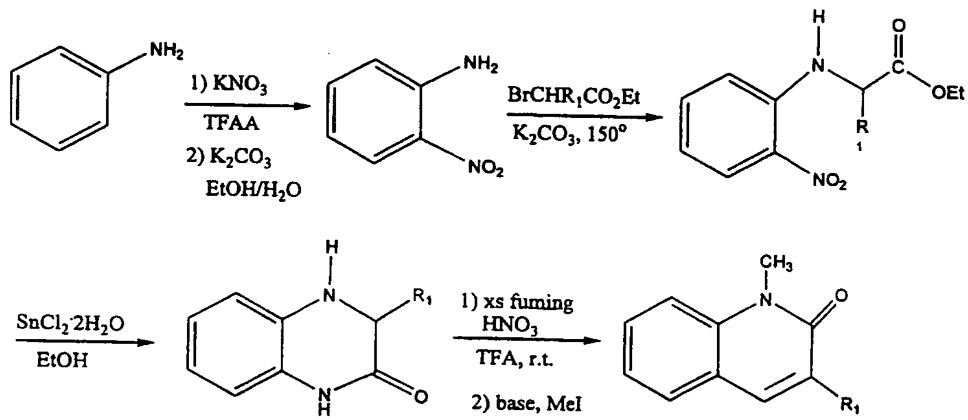
Scheme 1



Reaction scheme 2 follows procedures described by Kher, S.M. et al., Org. Chem., 60: 5838-42 (1995), in which substituted phenyl amines are reacted with KNO_3 to produce corresponding o-nitroanilines. The amino group is then alkylated by $BrCHR_1CO_2Et$, in which R_1 is as previously defined. Refluxing in the presence of tin (II) chloride effects ring closure via reductive condensation of the carbonyl and nitro moieties, producing 2-oxo-3-R_1-quinoxaline, which can be converted to the 3,4-dihydroquinoxaline form by treatment with fuming nitric acid. If desired, quinoxaline analogs may

be prepared by methylation at N_1 by reaction with methyliodide.

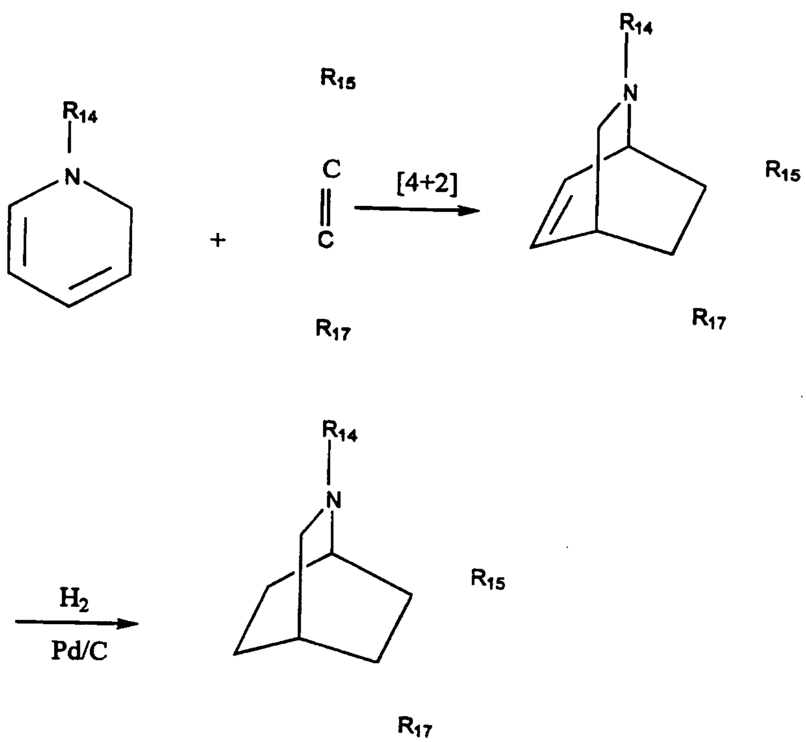
Scheme 2



General approaches to the synthesis of azabicyclo[2.2.2]octanes and azabicyclo[3.2.1]octanes are shown below in reaction schemes 3 and 4. Cycloaddition reactions, which form the basis for synthesis of these compounds, are extremely versatile in relationship to the configuration of the resultant ring systems and to their acceptance of various substituent groups.

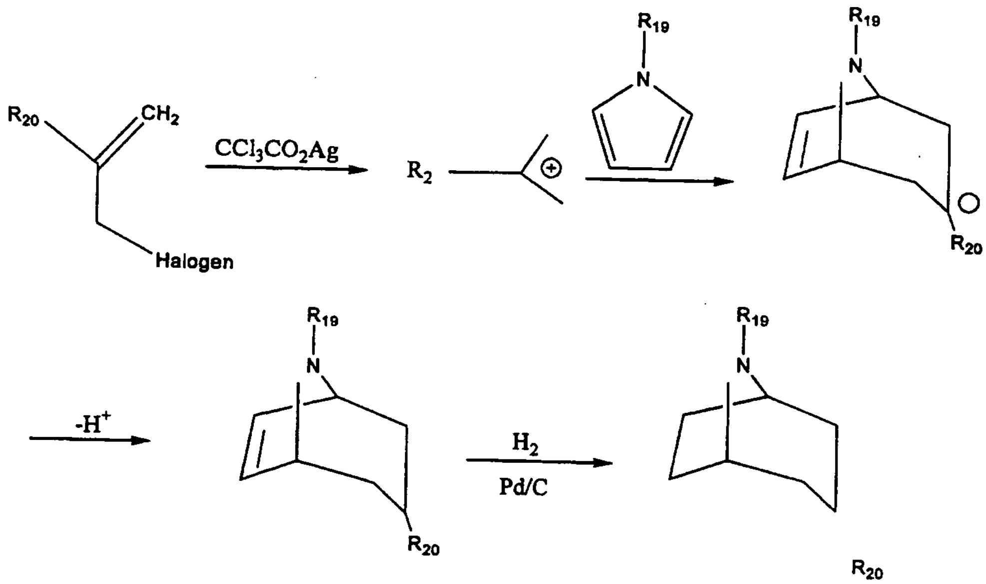
In reaction scheme 3, a 4+2 reaction, as described by Hoffman, Angew. Chem. Int. Ed., 12: 819-35 (1973), produces the [2.2.2]bicyclic ring system. Substitution of the nitrogen atom of the dihydropyridine moiety allows introduction of the R_{14} group, whereas R_{15} and R_{17} substituents are easily varied through the use of substituted ethylenes. R_{14}, R_{15} and R_{17} are as previously defined. A large number of appropriate starting materials are commercially available.

Scheme 3



In reaction scheme 4, which is carried out according to the procedure of Hoffman, Angew. Chem. Int. Ed., 23: 1-19 (1984), heating of an N-substituted pyrrole with an allyl cation intermediate (produced by extraction of an electron from the substituted allylhalide) produces the [3.2.1]bicyclic ring system. R_{19} and R_{20} are as previously defined.

Scheme 4



3-R_2-8-R_1-8-azabicyclo[3.2.1]octane

Pharmaceutically acceptable salts of the compounds described herein, which also have MDR reversing activity, e.g., the hydrochloride or sodium salts, may be prepared following procedures which are familiar to those skilled in the art.

The chemosensitizing pharmaceutical compositions of the present invention comprise one or more of the above-described compounds, as the active ingredient, in combination with a pharmaceutically acceptable carrier medium or auxiliary agent.

The composition may be prepared in various forms

for administration, including tablets, caplets, pills or dragees, or can be filled in suitable containers, such as capsules, or, in the case of suspensions, filled into bottles. As used herein, "pharmaceutically acceptable carrier medium" includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's Pharmaceutical Sciences, Fifteenth Edition, E.W. Martin (Mack Publishing Co., Easton, PA 1975) discloses various vehicles or carriers used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier medium is incompatible with the compounds of the invention, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

In the pharmaceutical compositions of the invention, the active agent may be present in an amount of at least 1% and not more than 95% by weight, based on the total weight of the composition, including carrier medium and/or auxiliary agent(s). Preferably, the proportion of active agent varies between 1% to 70% by weight of the composition. Pharmaceutical organic or inorganic solid or liquid carrier media suitable for enteral or parenteral administration can be used to make up the composition. Gelatine, lactose, starch, magnesium, stearate, talc, vegetable and animal fats and oils, gum, polyalkylene glycol, or other known excipients or diluents for medicaments may all be suitable as carrier media.

The compounds used in the practice of the invention may be administered using any amount and any route of administration effective for chemosensitizing multidrug-

resistant cells. Thus, the expression "therapeutically effective amount", as used herein, refers to a nontoxic but sufficient amount of the chemosensitizing agent to provide the desired effect against multidrug resistant cells. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the particular chemosensitizing agent, its mode of administration, and the like.

The chemosensitizing compounds described herein are preferably formulated in dosage unit form for ease of administration and uniformity of dosage. "Dosage unit form" as used herein refers to a physically discrete unit of chemosensitizing agent appropriate for the patient to be treated. Each dosage should contain the quantity of active material calculated to produce the desired therapeutic effect either as such, or in association with the selected pharmaceutical carrier medium. Typically, the chemosensitizing compound of the invention will be administered in dosage units containing from about 0.1 mg to about 10,000 mg of the agent, with a range of about 1 mg to about 1,000 mg being preferred.

The compounds of the invention may be administered orally or parenterally, such as by intramuscular injection, intraperitoneal injection, intravenous infusion or the like. The compounds of the invention may be administered orally or parenterally at dosage levels of about 0.1 to about 1,000 mg/kg and preferably from about 1 to about 100 mg/kg, of patient body weight per day, one or more times a day, to obtain the desired therapeutic effect.

Although the compounds described herein can be administered to any subject which is susceptible to development of multidrug resistance, the compounds are intended particularly for the treatment of cancer in humans.

The compounds of the invention will typically be administered from 1 to 4 times a day so as to deliver the above-mentioned daily dosage. Alternatively, dosages within these ranges can be administered by constant infusion over an extended period of time, usually 1 to 96 hours, until the desired therapeutic benefits have been obtained. However, the exact regimen for administration of the compounds and compositions described herein will necessarily be dependent on the needs of the individual patient being treated, the type of treatment(s) administered and the judgment of the attending physician.

The compounds of the invention can be used in various protocols for treating cancer patients. For example, these compounds can be used in a method for treating tumor cells in a patient requiring such treatment. This method would involve administering to a cancer patient a compound as described above in an amount effective to attenuate drug resistance in such cells.

These compounds can also be used in a method for treating hyperproliferative cells in a patient requiring such treatment, by administering the compound to a cancer patient in an amount effective to inhibit the proliferation of said cells.

The compounds of the invention can further be used in a method for potentiating an anticancer drug in a cancer patient undergoing chemotherapy. This method would involve administering an anticancer drug and at least one compound of those described above, in an amount effective to enhance the therapeutic efficacy of the anticancer drug. In this method, the anti-MDR compound may be administered to potentiate a natural product anticancer drug, an antitumor antibiotic anticancer drug, a natural or synthetic analogue of an antitumor agent, an antimitotic agent anticancer drug, or a natural or synthetic analogue of an antimitotic

agent. The antitumor antibiotic or the natural or synthetic analogue of an antitumor agent may be selected from the group consisting of daunorubicin, doxorubicin, idarubicin, mitoxanthrone, dactinomycin, bleomycin and plicamycin. The antimitotic agent or the natural or synthetic analogue of an antimitotic agent may be selected from the group consisting of topoisomerase inhibitors, microtubule inhibitors, etoposide, teniposide, amsacrine, topotecan, camptothecin, vinblastine, vincristine, vindesine, colchicine, paclitaxel, taxotere or other such agents that are subject to MDR. In this method, the MDR modulating compound can also be administered in combination with an additional compound effective to sensitize drug resistant tumor cells, the amount of the combination being effective to enhance the therapeutic efficacy of the anticancer drug. The additional compound may be selected from the group consisting of dihydropyridines, thioxanthenes, phenothiazines, cyclosporines, acridonecarboxamides, verapamil, cyclosporin A, PSC 833, tamoxifen, quinidine, bepridil, ketoconazole, megestrol acetate and estramustine.

In view of the beneficial effect on reversal of MDR produced by the compounds of the invention, it is anticipated that these compounds will be useful not only for therapeutic treatment after the onset of MDR, but also for MDR prevention in patients about to undergo chemotherapy for the first time. The above-noted dosages will be essentially the same whether for treatment or prevention of MDR.

Biological studies of the above compounds as MDR reversing agents have been performed. MDR reversal by means of chemosensitizing cells to anticancer drugs, such as actinomycin and daunomycin, was measured using the potentiation of cytotoxicity of known anticancer drugs in the presence of the compounds of the invention. The test procedures and results of these biological

studies are described below.

The following example sets forth test protocols for evaluating the MDR reversing activity of the modulating compounds described above, along with the test results. This example is provided for illustrative purposes only, and is not intended to limit the invention.

EXAMPLE

Evaluation of Reversal of MDR

Mediated by P-glycoprotein (P-gp) or MRP

The following cell lines were used in the studies described below: 1) MCF-7 human breast carcinoma cells; 2) MCF-7/ADR cells, an MDR subline which overexpresses P-glycoprotein (see, Fairchild, C.R. et al., Cancer Res., 47: 5141-5148 (1987)), but not MRP; and 3) human promyelocytic leukemia HL-60/ADR cells, which express MRP (see, March, W. et al., Cancer Res., 47: 4053-4057 (1986)), but not P-glycoprotein.

To test for reversal of P-glycoprotein-mediated MDR, MCF-7/ADR cells were seeded in 96-well tissue culture plates at approximately 15% confluency, and were allowed to attach and recover for 24 hours. The cells were then treated with the varying concentrations of the identified test modulator compounds alone or in the presence of 25 nM actinomycin D, or 1 μM daunomycin for 48 hours according to previously described procedures. See Smith, C.D., Oncology Res., 6: 211-218 (1994); and Smith, C.D. et al., Molec. Pharm., 47: 241-247 (1995). After 48 hours, cell survival was assayed using the sulforhodamine B (SRB) binding assay according to Skehan, P. et al., J. Natl. Cancer Inst., 82: 1107-1112 (1990). The percentage of cells killed is calculated as the percentage decrease in SRB binding compared with control cultures. Control cultures include equivalent amounts of ethanol (as the solvent control), which does not modulate the growth or drug-sensitivity of these

cells at doses used in these studies. Reversal of MDR is defined as the ability of the compound to potentiate the cytotoxicity of actinomycin D and/or daunomycin. To assess the toxicity of the compounds toward drug-sensitive cells, the effects of the test modulator compounds on the growth of drug-sensitive MCF-7 cells were determined using the same methods.

To test for reversal of MRP-mediated MDR, HL-60/ADR cells were treated with the varying concentrations of the identified test modulator compounds in the presence 0 or 2 nM vincritsine for 48 hours. The number of surviving cells was then determined using the CellTiter Aqueous assay system from Promega. The percentage of cells killed was calculated as the percentage decrease in 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-4-sulfophenyl)-2H-tetrazolium (MTS) metabolism, compared with control cultures. Reversal of MDR is defined as the ability of the compound to potentiate the cytotoxicity of vincristine toward the HL-60/ADR cells. The results of these tests are set forth in the following table. Reversal activity was scored on a scale of 0 - 5 (best activity = 5), taking into account the extent of reversal and the intrinsic cytotoxicity of the compound. On this scale, compounds with activity equal or superior to verapamil score 4, 4.5 or 5. The intrinsic cytotoxicity of the compounds towards MCF-7 cells is also indicated.

Compound	Cytotoxicity (% killed at 10 µg/ml)	Pgp reversal	MRP reversal
Dual antagonists			
verapamil	15	4.5	4
II-3	22	4.5	4
II-6	21	2	1.5
II-8	16	3	2.5
II-11	8	4	4.5
III-1	29	3	2
III-2	19	3	3
III-3	9	4.5	5
IV-3	36	2	2
IV-4	23	2	2
V-3	27	5	4.5
V-5	28	4.5	4.5
V-6	21	5	5
V-8	20	2.5	3
V10	18	3.5	3.5
Pgp-selective			
I-1	17	2	0
II-1	8	3	1
II-7	26	4	1
II-9	0	2	0
II-10	0	3	0
II-11	8	4	0
III-4	17	3	0
IV-1	18	4.5	0
IV-2	18	4	0
V-1	20	4.5	0.5
V-4	18	4	0
V-7	3	4.5	1.5
V-9	28	4.5	1.5
V-11	0	4.5	0.5

MRP-selective			
I-3	7	0	4.5
I-2	3	0	4
II-2	39	1	3
II-4	12	0	2
II-5	0	0	4
V-2	4	2	4.5
V-12	16	2.5	4.5
V-13	11	1	4.5

As can be seen from the foregoing test results, each compound significantly increases the toxicity of actinomycin D toward MCF-7/ADR cells and/or vincristine toward HL-60/ADR cells. The data demonstrate that the test compounds are either: 1) selective inhibitors of P-glycoprotein function; 2) selective inhibitors of MRP function; or 3) dual inhibitors of both transporters. Most of the compounds tested have low intrinsic cytotoxicity, (<20% of cells killed by doses of 10 micrograms/ml), as is desired for an MDR modulator.

Because of their ability to inhibit MDR, these compounds will have utility in the therapy of cancer.

Although the present invention has been described and exemplified in terms of certain preferred embodiments, other embodiments will be apparent to those skilled in the art. The invention is, therefore, not limited to the particular embodiments described and exemplified, but is capable of modification or variation without departing from the spirit of the invention, the full scope of which is delineated by the appended claims.

WHAT IS CLAIMED IS:

1. A method for potentiating an anticancer drug in a cancer patient undergoing chemotherapy involving administration of said anticancer drug, said method comprising administering to said patient at least one anti-MDR compound, as described in the foregoing specification, in an amount effective to enhance the therapeutic efficacy of said anticancer drug.

2. A method as claimed in claim 1, wherein said compound is administered to potentiate a natural product, anticancer drug.

3. A method as claimed in claim 1, wherein said anticancer drug is an antitumor antibiotic.

4. A method as claimed in claim 3, wherein said antitumor antibiotic is selected from the group consisting of daunorubicin, doxorubicin, idarubicin, mitoxanthrone, dactinomycin, bleomycin and plicamycin.

5. A method as claimed in claim 1, wherein said anticancer drug is a natural or synthetic analogue of an antitumor agent selected from the group consisting of daunorubicin, doxorubicin, idarubicin, mitoxanthrone, dactinomycin, bleomycin and plicamycin.

6. A method as claimed in claim 1, wherein said anticancer drug is an antimitotic agent.

7. A method as claimed in claim 6 wherein said antimitotic agent is selected from the group consisting of topoisomerase inhibitors and microtubule inhibitors.

8. A method as claimed in claim 6, wherein said antimitotic agent is selected from the group consisting of etoposide, teniposide, amsacrine, topotecan,

camptothecin, vinblastine, vincristine, vindesine, colchicine, paclitaxel and taxotere.

9. A method as claimed in claim 1, wherein said anticancer drug is a natural or synthetic analogue of an antimitotic agent selected from the group consisting of etoposide, teniposide, amsacrine, topotecin, camptothecin, vinblastine, vincristine, vindesine, colchicine, paclitaxel and taxotere.

10. A method as claimed in claim 1, wherein said compound is administered in combination with an additional compound effective to sensitize drug resistant tumor cells, the amount of said combination being effective to enhance the therapeutic efficacy of said anticancer drug.

11. A method as claimed in claim 10, wherein said additional compound is selected from the group consisting of dihydropyridines, thioxanthenes, phenothiazines, cyclosporines and acridonecarboxamides.

12. A method as claimed in claim 10, wherein said additional compounds is selected from the group consisting of verapamil, cyclosporin A, PSC 833, tamoxifen, quinidine, bepridil, ketoconazole, megestrol acetate and estramustine.